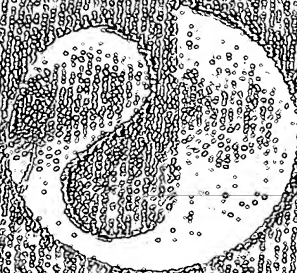


10/677,956/Declaration, Torston B. Holling

January 7, 2007

EXHIBIT# 4



Pharmacia

Pharmacia Genetic Engineering Inc.

NO. 51

6.29 Cloning of Capsid PCR Fragments

PCR reactions of 6.28 #1 690-691 360 bp full size
all from SF.cDNA 190 bp ?

#3 690-694 233 bp

#5 891-693 215 bp

set up remainder of PCR reaction on 5% PAGE prep gel
1 day \Rightarrow EtBr stain - cut out above bands

4 bands coil gel slice - electroelute 15' 1 watt
20' 3 watts

collect DNA

ϕ -CHCl₃ extract

EtOH ppt -80° 1 hr

spin out DNA

set up kinase reactions

pellet up in 76 μ l H₂O

2 μ l 1m ATP

2 μ l PNE

10 μ l link kinase

37° 1 hr

ϕ -CHCl₃ extract

EtOH ppt -80° 1 hr

pellet up in 20 μ l H₂O

1/2 of pellet amt into ligation reactions

6.5 μ l H₂O

10 μ l DNA

0.5 μ l PCR small cut

2 μ l 10x ligase kinase buffer

1 μ l T₄ ligase

rt o/n

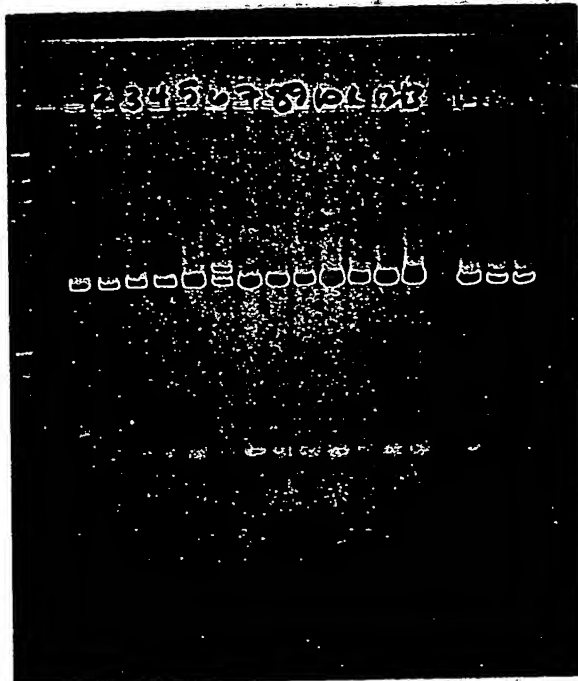
6.30 Transformation

IMB3 cells 4x PCR-PCR reactions

plate cells on XGAMP plates

7.1 replate as not many x-formants

7.2 re-transform ligations - IMB3 to increase #'s



7.4

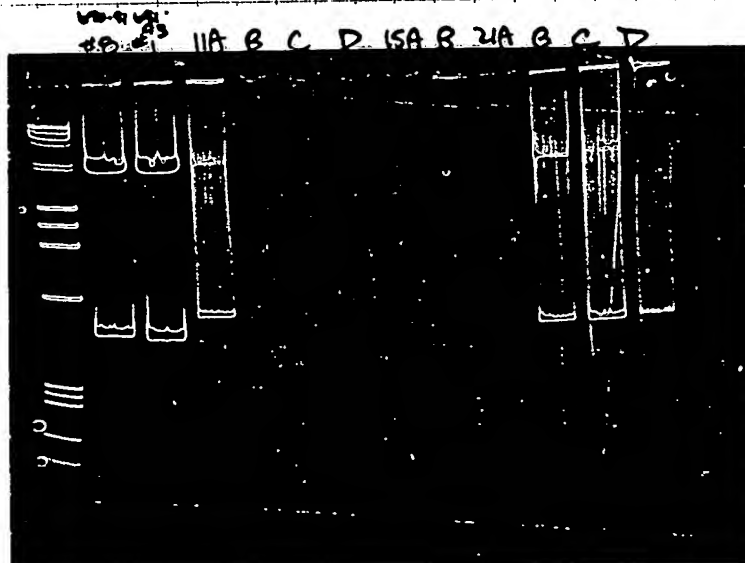
PHOTOMETRY/DNASCAN4U

07/09/90 11:01

ID ABS

260.0nm	0.209A	691473 #1	1.045
260.0nm	0.146A	69106911 #8	0.73

260	0.391	690694 #9
-----	-------	-----------



7.9

7.1 mini preps on capsid fragments into PCR 18

CYNDIES preps

PCR 1 top = mprep #1 → 3 690.691
 PCR 1 btm = mprep #4 → 9 690.691
 PCR 3 = mprep #10 → 18 690.694

see her notebook for results

→ only positive = PCR 1 btm #9
 690.691

7.2 more mini preps

PCR 1 top = #1 → 3 690.691
 PCR 5 = #1 → 13 691.693

see photo at right

→ only positive = PCR 5 #1
 691.693

7.4 more mini preps

#1 → 9 PCR 1 top 690.691
 #1 → 9 PCR 3 690.694

see cyndies notebook -
 identified positive
 PCR 3 690.694 #9

7.5 maxi preps on

690.691 btm #8
 691.693 (PCR 5) #1

7.9 maxi prep on

690.694 #9

7.8 mini preps repeated on cyndies of 7.7 →

positive 690.691 #15
 #21
 690.694 #11

but colonies = double pick
 replat XGAMP (all white)
 choose separates:
 remini prep. (A → D and)

PCR II digest 4 x mpreps of each (ISCID did not grow)

choose 11A 690.694
 213 690.691

7.10 maxi prep

ABS

260.0nm 0.156A 11A 780ng/ul
 260.0nm 0.391A 213 1.955ug/ul

7.13 remini prep #15 690.691 isolates → EFGH IJ

see cyndies notebook

choose 2 for maxi prep

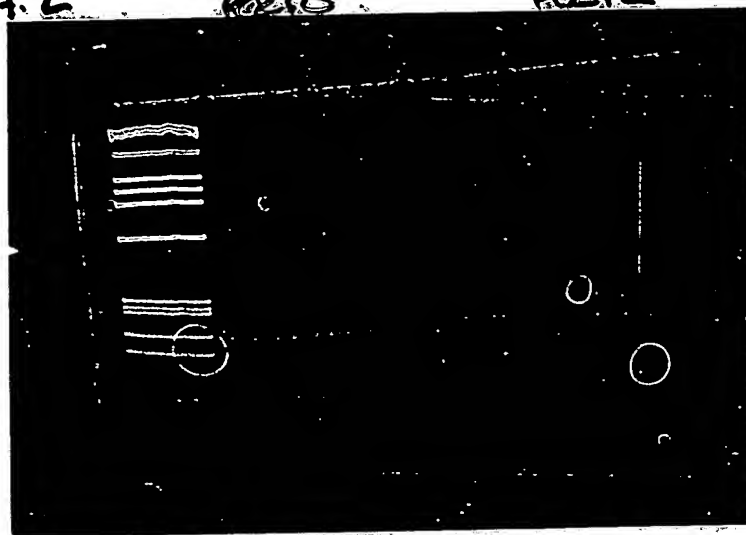
7.16 maxi prep of #15 E : 15H 690.691

Page 67 = continuation of
 cloning attempts

7.2

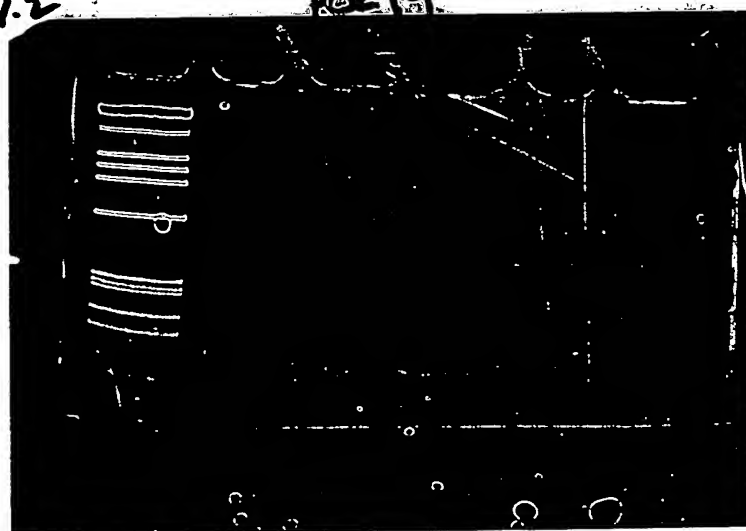
REC 10

REC 12



7.2

REC 14



7.2 Cloning of PCR fragments from 687-688 oligos 0000

PCR 10 687-688 fragments of 230 bp (1. than predicted 117 bp)
 PCR 12 682-687 250 bp fragment (not as predicted)
 PCR 14 683-687 150 bp fragment (smaller than predicted)

run remainder of PCR reaction on 5% PAGE prep gels

cut out 1 bands 230 bp PCR 10
 250 bp PCR 12
 150 bp PCR 14

elute 20 1 watt
 20 3 watt } CHCl₃ extract
 EtOH ppt. -80° 1 hr

kinase 2ul 10x ATP
 2ul Pfu
 10ul 10x linc-kin buffer
 70ul H₂O to pellets

37° 1/2 hr CHCl₃ extract
 -80° 1 hr EtOH ppt. res. up in 20ul
 1/2 sec 1/2 -20

ligate to PCR 18

10ul DNA
 6.5ul H₂O
 0.5ul Pfu SmaI
 2ul 10x ligase kinase buffer
 1ul T4 ligase

part

7.3 Transformation into JM83 cells plate onto XbaI Amp plates

Results show no whites. ∴ few colonies. NO colonies for PCR 14.

18 replate transformations to increase colony numbers.

7.9 minipreps on PCR 10 PCR 12 } Pvu II digested
 #1-9 #1-9 → SEC CYDIES NB
 results show no positive colonies.

7.12 repeat of more PCR 10 PCR 12 minipreps

again no positive colonies → redesign clones!

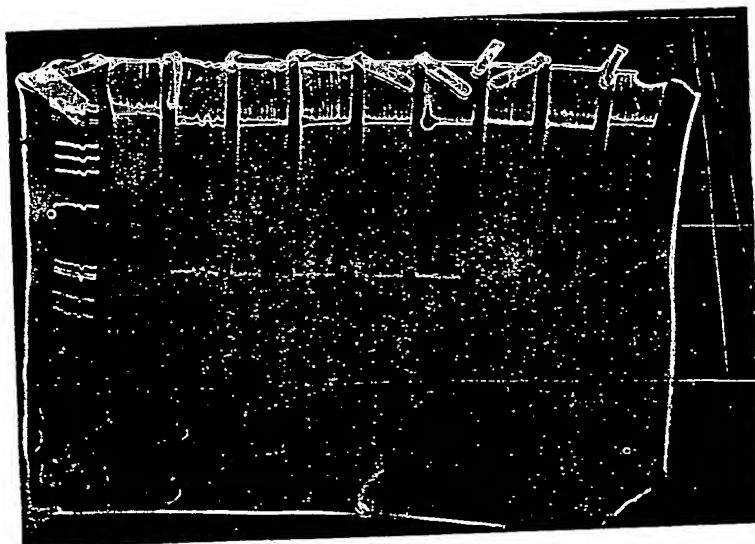
Attempts to re-isolate 693-691 clones

only one previously identified
691-693-#1

sequence analysis (p 79) shows shorter than
expected - repeat from ligation to
identify more clones

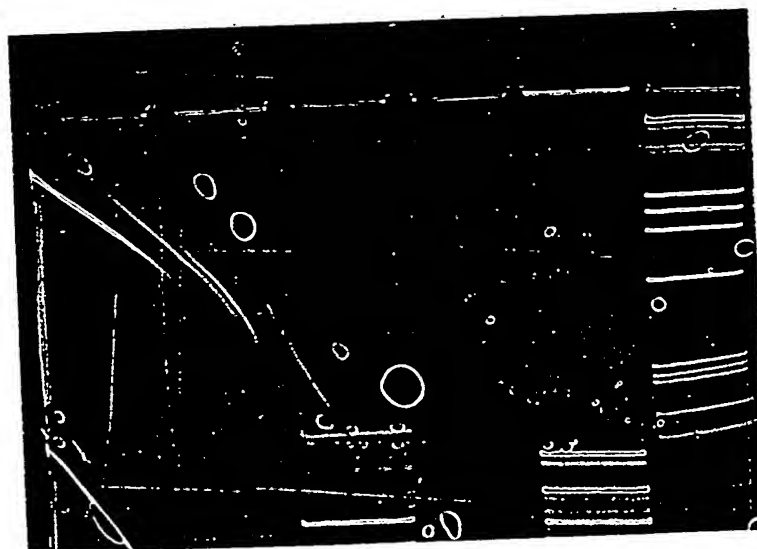
SEC CINDIES Notebook for \times formations
mini preps

Attempts RVI digests of mpreps



7-19 CYNs mpreps

no positives identified



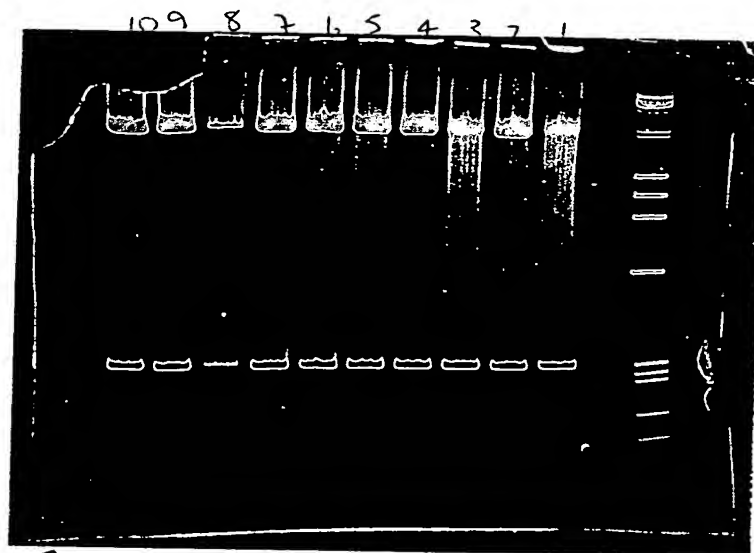
7-25

only PRS.1
good 5

7.23

more 691693 mpreps
 replate PCR 5 xformation mix
 choose white colonies
 grow white clones → in Amp
 duplicate to lower more mprep
 PvuII digest

Results



7.25

7.24 Repeat PCR clonings of 690691 Full capsid = 691693

Use 1st round PCRs from 6.27 - page 63

*1-14 stored at -20°

resetup #1 690691 S9 cDNA
 #5 691693 S9 cDNA
 #6 691693 PCR cDNA

new PCR = 5

		AB	TAQ	H ₂ O	690	691	693	1
1	*1 redo	50	10	25.6	2.76	1.61	-	-
2								
3								
4								
5	*5 redo			26.69			17	
6								
7	*6 redo			26.69				
8								
9								
10								

results
 ←

EVE FINE 11

① CHCl₃ ECTH ppt up in 400ul H₂O → prep gel

35S reactions

626.627	590 BS primer	
626.627	591 BS primer	
626.627	626 primer	
626.627	627 primer	
628.629	628 primer	
628.629	652 primer	
2.F.	674 primer	674.675 = 2.F.
2.F.	675 primer	
571.623 #10	571 primer	

Results show priming with all -

590/591 = shadow bands

626 = light sequence

others look good

7.3 35S sequences entered into VAX

200-300 bp. for each combo.

Processing data of 30JUN90 ALF.

- 1] processed ok, but all overlapping peaks
- 2]
- 3] processed ok, but overlapping peaks
- 4]
- 5 processed - 225+ bases - wild pattern : can continue
- 6 could not compute peak width. Given peaks 1 - off scale?
- 7 processed - 240 bases
- 8 peak width off - could not process
- 9 processed, but overlap peaks
- 10 could not compute peak width.

peaks w/ reverse primer.

6-27-90

35S sequencing of HepC clones - use internal primers.

To Denature					5'ug
626, 627 (#46)	4 tubes	1ul = 1.21ug		4.13	
628, 629 (#16)	2	1ul = 1.56ug		3.2	
2, F. (#39)	2	1ul = 0.43ug		11.6	
571, 623 (#10)	1	1ul = 1.98ug		2.5	

To each tube add 2ul 2N NaOH.

leave rt 10'

+ 3ul NAC

+ 100ul ETOH + 20ul H₂O -70° 1HR wash

Follow Cynclis procedure using Pharmacia kit.

run on 8% Gels.

short = BBdfe

long = XCdfe + 3/4 of 2nd R

6-29-90

ALF sequencing to complete clone information.

PCR 2#2 1ul = 0.465ug

PCR 2#7 1ul = 0.74ug

626, 627 1ul = 1.21ug

628, 629 1ul = 1.56ug

302uN ALF

(problems w/ 29 JUN 90 ALF)

#			QNA	H ₂ O
1	PCR 2 #2	universal	10	-
2	PCR 2 #2	reverse	10	-
3	PCR 2 #7	universal	6.75	3.25
4	PCR 2 #7	reverse	6.75	3.25
5	626, 627	universal	4.13	7.87
6	626, 627	reverse	4.13	7.87
7	628, 629	universal	3.2	6.8
8	628, 629	reverse	3.2	6.8
9	PCR 2 #2	universal	10	-
10	PCR 2 #2	reverse	10	-

resulting reported sequences = only 2!

626, 627 - U. Seq

628, 629 - U. Seq

571623 clone

571623-571.Seq Length: 294 July 3, 1990 15:53 Check: 3145

```
1 AGTGGGGAGA ACTTTCCTTA CCGGTAGCG TACCAAGCCG CCGTGTGCGC
51 TAGGGCTCAA GCCTCCCCCA TCGTGGGACC AGATGTGGAA GTGTTTGATC
101 CGCCTTAAAC CCACCTTCCA TGGCGCCAAC ACCCTTGGAT ACAGACTGGG
151 CGCTGTTCAG AATGAAGTCA CCCTGACGCA GGGAAATACC AATACATCA
201 TGACATGGGG TACCGAGCTC GAATTCGTAA TCAATGCATA GCCTTTCTCT
251 GTGTGAATTG TTATCGCTC ACAATCCAG ACAACATACG AGCC
```

571623-10f.Seq x 571623-571.Seq July 3, 1990 10:30 ..

```
74 AGTGGGGAGA ACTTTCCTTA CCGGTAGCG TACCAAGCCG CCGTGTGCGC 123
|||||
1 AGTGGGGAGA ACTTTCCTTA CCGGTAGCG TACCAAGCCG CCGTGTGCGC 50
124 TAGGGCTCAA GCCTCCCCCA TCGTGGGACC AGATGTGGAA GTGTTTGATC 173
|||||
51 TAGGGCTCAA GCCTCCCCCA TCGTGGGACC AGATGTGGAA GTGTTTGATC 98
174 TCCGCTTAAACCCACCTTCCATGG GCCAACCCCCCTGTATACAGACT 222
|||||
99 TCCGCTTAAACCCACCTTCCATGG GCCAACCCCCCTGTATACAGACT 147
223 GGGCGCTGTTCAGAAATGAAGTACCCCTGACGSCACCCCAATCACCAGATACA 273
|||||
148 GGGCGCTGTTCAGAAATGAAGTACCCCTGACGSCAGGGAAATCACCAGATACA 197
273 TCAATGACATGC 283
|||||
198 TCAATGACATGC 208
```

see page 37

571623 clone info

- confirms seq. A.

alteration from previous seq's.

21f1-U.Seq x 21f1-675.Seq July 3, 1990 10:35 ..

```
134 agcgtagcggtaggtcctcaccgaatcaaccctacctaactgaccttggccagg 183
|||||
1 AACGTACGCTGTGCTCCACCGAATCAACCTTACCTACTGCTTGGCCGAG 49
184 cttgccaccgaggggtttggcagctcctcaacttccggcattacgggagg 237
|||||
50 CTTGCCACCAAAAGTGTGTCAGCTCCCTCAACTTCCGCAATTACGGGCGA 99
|||||
234 caatacgacaacatcctctgagcgccgcccccttctggctg.ccccccgaact 282
|||||
100 CAATACGACAACATCTCTGAGCCCGCCCCCTTCTGGCTGCCCCCGGACT 149
283 ccgagcttgagtgacctattcttccatgccccccctggagggagacctggagg 337
|||||
150 CCGAGCTTGAGTCTTATCTTCCATGCCCCCCCCCTGGAAGGAGGCTGGGGGA 199
333 t.cggatctcagcgagcgggtcctggtcgacgggtcagtagtggggg 376
|||||
200 TCCGATCTCAGCGACGGGTCAATGCTCAGGGTCAATAGTGGGGC 244
```


628629-U.Seq x 628629-628.Seq July 5, 1990 11:03 ..

132 TTTGGAGAACCTCGTAATACICAATGCAGCATCCCTGGCCGGGACGCACG 181
 2 TTTGGAGAACCTCGTAATACICAATGCAGCATCCCTGGCCGGGACGCACG 51
 182 GTTTTGTGTCTTCCCTCGTGTTCCTCTCTGTTTGGGTGGTATCTG 225
 52 GTTTTGTGTCTTCCCTCGTGTTCCTCTCTGTTTGGGTGGTATCTG 95

628629-628.Seq x 628629-R.Seq July 6, 1990 07:48 ..

```

41 CGGGACGCGACGGTTTTGTGTCTTCCCTCGTGTTCTTCTGTTTTGCGTGTT 90
265 CCGGACGCGACGGTTTTGTGTCC.TCCTCGGT.CTCGT.TTGTGCGTT 120
91 ATCTGAAGGGTAGGTGGGTGCCCGGAGCGGTCTACGCCCTCTACGGGATG 140
319 A.CTGAAGGGTAGGTGGGTGCCCGGAGCGGTCTACGCCCTCTACGGGATG 175
141 TGGCCTCTCCTCCTGCTCCTGCTGCGGTGCCTCAGCGGGCATACGCACT 190
172 TGGCCTCTCCTCCTGCTCCTGCTGCGGTGCCTCAGCGGGCATACGCACT 123
191 GGACACGGAGGTGGCCGCGTCGTGTGGNGGCGTTGTTCTTGTGCGGGTTAA 240
122 GGACACGGAGGTGGCCGCGTCGTGTGCGGCGTTGTTCTTGTGCGGGTTAA 73
241 TGGCGCTGACTCTGTGCGCCATATT 265
72 TGGCGCTGACTCTGTGCGCCATATT 48

```

628629-U.Seq x 628629-R.Seq July 5, 1990 11:02 ..

```

171 CCGGACGACACGGTTTGTGTCCCTCCTCGTGTCTTCTGTCTTTCGGTGGT 220
    | | | | | | | | | | | | | | | | | | | | | | | | | | | |
225 cCGGACGACACGGTTTGTGTCCCTCCTCGTGT..CTCTG.TTTGGGTGGT 180

221 A 221
    |
179 A 179

```

cannot identify homology with 652 sequence
link up other seqs into 628629 - F. seq.

2590

0.00

Sequence Analysis of 628629 clone

from 355 sequence - 303W ALF

628629-U.Seq Length: 225 July 2, 1990 11:34 Check: 8218

1 TAATACGACT CACTATAGGG CGAATTGGGT ACCGGGCCCC CCTCGAGGT
 51 CGACGGTAIC GATAAGCTTG ATATCGAATT CCTGCAGCCC TCGTTGTGA
 101 TGATGCTACT CATATCCCAA GCGGAGGCGG CTTTGGAGAA CCTCGTAATA
 151 CTCAATGCAG CATCCCTGGC CCGGACGCAC GGTITTTGTGT CCTTCCTCGT
 201 GTTCTTCTGT TTTCGTGGT ATCTG

ALF

628629-652.Seq Length: 247 July 3, 1990 15:42 Check: 6340

1 CATTCGTAT TACGAGGTTT TCCAAAGCCG CCTCCGCTTG GGATATGAGT
 51 AGCATCAICC ACAAGCAGGG CTGCAGGAAT TCGATATCAA GTTATCGAT
 101 ACCGTGACCC TCGAGGGGGG GCGCGTACC CAATTCGCCC TATAGTGAGT
 151 CGTATTACGC GCGCTCACTG GCGCTCGTT TACAACGTGG TGACTGGGAA
 201 AACCCTGGCG TTACCCAACI TAATCGCCTT GCAGCACATC CCCCIT

355

628629-628.Seq Length: 265 July 3, 1990 15:39 Check: 3747

1 GTTGGAGAA CCTCGTAATA CTCAATGCAG CATCCCTGGC CCGGACGCAC
 51 GGTITTTGTGT CCTTCCTCGT GTTCTTCTGT TTTCGTGGT ATCTGAAGGG
 101 TAGGTGGGTG CCCGGAGCGG TCTACGCCCT CTACGGGATG TGGCTCTCC
 151 TCTGTCTCT GTTGGCGTTG CCTCAGCGGG CATACGCACT GGACACGGAG
 201 GTGGCCGCGT CGTGTGGNGG CTTTGTCTT GTGGGTTAA TGGGCTGAC
 251 TCTGTGCGCA TATTA

355

Previous Sequence Results page 58

628629-1

628629-N4EC

Bestfit Analysis

626627-590.Seq x 626627-626.Seq July 5, 1990 15:32 ..

165 ctgagctcggacctgtcctgtcttccagatcacagcttt 203
 1 CTGAGCTCGGACCTGTCTGTCTTCCAGATCACAGCGTT 39

b26?

626627-U.Seq x 626627-591.Seq July 6, 1990 08:13 ..

72 TATCGAATTCCTGCGCCCCCGCAAAGAGCGTGTGTGGCCCGGTATATTG 121
 1 TATCGAATTCCTGCGCCCCCGCAAAGAGCGTGTGTGGCCCGGTATATTG 50
 122 CTTCACCTCCAGCCCCGTGGTGGTGGGAACGACGACAGGTGCGCGCTA 171
 51 CTTCACCTCCAGCCCCGTGGTGGTGGGAACGACGACAGTGTGCGCGCTA 99
 172 CCTACAGCTGGGGTGCAAATGATACGGATGTCTTCGTCTTACACACAC 220
 100 CCTACAGCTGGGGTGCAAATGATACGGATGTCTTCGTCTTACACACAC 149
 221 CAGGC 225
 150 CAGGC 154

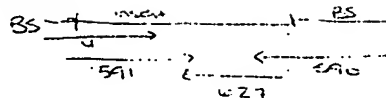
626627-U.Seq x 626627-Nybc.Seq July 5, 1990 15:55 ..

91 CCGCAAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCAGCCCCGTG 140
 1 CCGCAAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCAGCCCCGTG 50
 141 GTGGTGGGAACGACCGACAGGt...GCGCGCTACCTACAGCTGGGGTGC 187
 51 GTGGTGGGAACGACCGACAGGTCGGGCGCGCTACCTACAGCTGGGGTGC 100
 188 AAATGATACGGATGTCTTCGTCTTAAACAACACACAGGC 225
 101 AAATGATACGATGTCTTCGTCTTAAACAACACACAGGC 138

626627-Nybc.Seq x 626627-591.Seq July 6, 1990 08:15 ..

276 GNTATTTANGGAAGCAATCAGTGGGGCAGAKCAAGGTGTGTGTGGCGN
 299 GATATTGCG.AAGCAATCAGTGGGGCAGAGCAAGG GTTGTGTGGCGA
 226 CCTCCGATGACNCAAGGGGGNGCTCCGACACTTGTGTGAATCCAGT
 250 CCTCCGATGACACAAGGGGGCG.TCCGACACTTGTGTGAATCCAGT
 176 GTTCATCCAGGTACAACCGAATCAATTGCCAGCGGTGGGCTGGTGT
 201 GTTCATCCAGGTACAACCGAATCAATTGCCAGCGGTGGGCTGGTGT
 126 .TAAAGGAYGAAGACATTCATTTGCACCCAGCTGTAGGTAGG
 151 GTAAGGACGAAGACATCCGATATTCATTTGCACCCAGCTGTAGGTAGG
 77 GCGGACCTGTGGTTCGTCCACACACAGGGGCTGGGAGTGAAGC
 101 GC...ACTGTGGTTCGTCCACACACAGGGGCTGGGAGTGAAGC
 27 ATACCGGGCCACACAGCTTTTGGCG 1
 55 ATACCGGGCCACACAGCTTTTGGCG 39

Organization



7-6-90

Construction/organization of sequence information

0000

626627 clone from NYBC

626627-590.Seq Length: 203 July 3, 1990 15:20 Check: 760

1 actaaagggg acaaaagctg gagctccacc gaggtagcgc gctctagaac
 51 tagtggatcc cccaatgttc tggtaggagt ggaatgaggcc ggtggacaag
 101 gctggcaggg tcgtgaaaga ccacgggaag acctgccact gtgtggtggc
 151 acagcagcaa cgggctgagc tcggacctgt cctgtcttcc agatcacagc
 201 ttt

626627-626.Seq Length: 192 July 3, 1990 15:27 Check: 1506

1 CTGAGCTCGG ACCTGTCTTG TCTTCCAGAT CACAGCGTTC GCCCGCTCC
 51 AGTTGCAGGC CGCTTCCAGC CTGTGCTCGA CCGCTCCAGG GTACATCTTG
 101 ACTTTGAATA TGGTGTAATT GATGTACAA GATAGTGC AAAGCCTATA
 151 CGGGTACTCG ACCAIGCAAC TGGTGTAAT CCAGGACCGA AG

626627-627.Seq Length: 301 July 3, 1990 15:31 Check: 6567

1 AGGCCGGTGG ACAAGGCTGG CAGGGTCGTG AAAGAACAGG GAAGGACCTG
 51 CCACTGTGTG TGGACAGCAG CAACGGGCTC AGCTGGGACC TGTCCCTGTC
 101 TTCCAGATCA CAGCGTTCGC CCCGCGTCCA GTTGACGGCC GCTTCCAGCC
 151 TGTGCTCGAC CCTTCCACAG TACATCTTGA CTTGAATAT GGTGTAATTG
 201 ATGGTACAA GATAGTGC CAAGCCTATAC GGGTAGTCCA CCAIGCACCT
 251 GGGTGTAAAT CTGGGACCGG AGCCGACCGG AGAGTATGTG GTTCCGATA
 301 T

626627-591.Seq Length: 292 July 3, 1990 15:24 Check: 2620

1 TATCGAATTC CTGAGCCCC CGCAAAGAGC GTGTGTGGCC CGGTATATTG
 51 CTTCACCTCC AGCCCCGTGG TGGTGGGAAC GACCGACAGT GCGCGCTTAC
 101 CTACAGCTGG GGTGCAAAATG ATACGGATGT CTTCGTCTT ACACAACACC
 151 AGGCCACCGC TGGGCAATTG GTTCGGTGT ACCTGGATGA ACTCAACTGG
 201 ATTACCAAA GTGTGCGGAC GCGCCCTTGT GTATCGGAG GGGTGGGCAA
 251 CAACACCTTG CTCTGCCCCA CTGATTGCTT CGCAAAATAT GG

626627-U.Seq Length: 225 July 2, 1990 11:34 Check: 6325

1 TAATACGACT CACTATAGGG CGAATGGGT ACCGGGCCCC CCTTCBAGGT
 51 CGACCGTATC GATAAGCTTG ATATCGAAT CTTCAGGCC CGGCAAGAG
 101 CGTGTGTGGC CGGTATATT GTTCACTCC CAGCCCCGTG GTGGTGGGAA
 151 CGACCGACAG GTGCGGCGCT ACCTACAGCT GGGGTGCAAA TGTACGGAT
 201 GTCTTCGTCC TTAACAACAC CAGGC

626627 organization HCV

Hcv.Seq x 626627-591.Seq July 6, 1990 09:29 ..

```
144 CCCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT 193
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
19 CCCGCAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCCAGCCCCGT 69
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
194 GGTGGTGGGAACGACCGACAGGTGCGGGCGCGCCACCTACAGCTGGGGTG 243
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
69 GGTGGTGGGAACGACCGACA...GTGCGCGCGCTACCTACAGCTGGGGTG 114
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
244 AAAATGATACGGACGCTTCGTCCTTAACAATACCGAGGCCACCGCTGGG 292
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
115 CAAATGATACGGATGTCTTCGTCCTTAACAACACCGAGGCCACCGCTGGG 164
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
293 CAATTGGGTTGGGTTGTACCTGGATGAACICAACTGGATTACCAAGTGT 342
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
165 CAATTGGGTTGGGTTGTACCTGGATGAACICAACTGGATTACCAAGTGT 214
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
343 GCGGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCAC 392
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
215 GCGGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCAC 263
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
393 TGCCCCACTGATTGCTTCCGCAAGCATCG 422
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
264 TGCCCCACTGATTGCTTCCGCAAAATCGG 292
```

compare
confirm

Hcv.Seq x 626627-Nybc.Seq July 6, 1990 09:30 ..

```
145 CCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT 194
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1 CCGCAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCCAGCCCCGT 50
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
195 GTGGTGGGAACGACCGACAGGTGCGGGCGCGCCACCTACAGCTGGGGTG 244
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
51 GTGGTGGGAACGACCGACA...GTGCGCGCGCTACCTACAGCTGGGGTG 100
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
245 AAATGATACGGACGCTTCGTCCTTAACAATACCGAGGCCACCGCTGGGCA 294
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
101 AAATGATACGRATGTCTTCGTCCTTAACAACACCGAGGCCACCGCTGGGCA 150
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
295 ATTGGTTGGGTTGTACCTGGATGAACICAACTGGATTACCAAGTGTGC 344
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
151 ATTGATTGGGTTGTACCTGGATGAACICAACTGGATTACCAAGTGTGC 200
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
345 GGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCAC 394
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
201 GGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCAC 250
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
395 CCCCAGTGAATTGCTTCCGCAAGCATCCGGAGCCACATACTCTCGGTCG 444
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
251 CCCCAGTGAATTGCTTCCGCAAGCATCCGGAGCCACATACTCTCGGTCG 300
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
445 G 445
      |
301 G 301
```

0000

```

- 421. CCGACGCCACATACTCTCGGTGCGGCTCCGGTCCCAGGATCACACCCAGG- 470
      ||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
996 CCGAAGCCACATACTCTCGGTGCGGCTCCGGTCCCAGGATTACACCCAGG 942

471 TGCCTGGTCGACTACC CGTATAGGCTTTGGCAATTATCCTTGTAACCATCAA 520
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
946 TGCATGGTCGACTACC CGTATAGGCTTTGGCACTATCCTTGTAACCATCAA 892

521 CTACACCATATTTAAAAICAGGAATGIACGTGGGAGGGGGTCGAACACAGGC 570
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
896 TTACACCATATTTCAAAGTCAGGAATGIACGTGGGAGGGGGTCGAGCACAGGC 842

571 TSSAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTGSAAGAC 620
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
846 TSSAAGCGGGCTTCAACTGGACGCGGGGCGAACGCTGTGATCTGSAAGAC 792

621 AGGGACAGGTCGAGGCTCAGCCCGTTACGTGACCACTACACAGTGGCA 670
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
796 AGGGACAGGTCGAGGCTCAGCCCGTTGCTGCTGTCCACACACAGTGGCA 748

671 GGTCTCTCCCGTGITCTTTCACAACCTTACCAGCCTTGTCACCGGCT 718
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
747 GGTCTCTCCGTGTTCTTTACGACCTTCCAGGCTTGTCACCGGCT 700

```

missing
confir

601 AACGTTCGCACTCGAAGACAGGGACAGGTCGAGGCTCAGCCCGTCTACTG 630
 1000 aaagcgtgctgctcggagacggaacagggtccgagctcagcccgcttgctg 958
 651 CTG.ACCACTACACAGTGGGAGGTCTCCCGTGTCTCTTCACAAACCTAC 699
 961 ctgagccaccacacagtgggcaggctcctccgtggtctttcacgaccctgc 968
 700 CAGCTTGTCCACCGGCTTCATCCACCTCCACCAAGACATTGTGG 744
 801 cagcctgtccaccggcctgctccacctccaccggaacattgagg 852

-pat = confirmed. A w/HCV

Computer Assembly (cont'd)

21F1 - F seq

21F1 - U #1-272
 21F1 - 675 #137-137
 21F1 - 674 #275-39
 21F1 - r #56-1

Δ 198 del A, +T
 add GG
 del AC
 add t

HCV pair

5600-6600

524bp
 95.029 / 5

628629 - F seq

628629 - U #1-225
 628629 - 628 #96-217
 628629 - r #1-2052

HCV pair

850-1231

382 bp. home
 94.76 / 5mi

626627 - F seq

626627 - U #1-225
 626627 - 591 155-end
 626627 - 627 #1-297
 626627 - 590 #1-85

HCV pair

144-900

601 bp. home
 94.509 / 5mi

w/o Computer Assembly of Sequence Information

Notes on organization and Bestfit Analysis

571623 - 10F seq.

add sequence info on to end of already established file
pair w/ HCV

3300 - 3600

243 bp hc
97.1% sim

62126 - 5F seq

from 5U

121 - del A

150 - w = A

129 - add del G

207 - add A

HCV pair

5959 - 6200

182 bp hc
97.2% sim

628652 - 2F seq

from 2U

no A

HCV pair

852 - 959

108 bp hc
98.148

617652 - 4F seq

from 571623 - 4U

94 del C

168 T → C

HCV pair

789 - 960

172 bp hc
97.093

7/19/90

ALF sequencing

9 JULY 90

clones

1	654 687	250bp	#6	universal	5ug
2	654 687	250bp	#6	reverse	10ul
3	654 687	250bp	#1	universal	10ul
4	654 687	250bp	#1	reverse	10ul
5	654 687	120bp	#6	universal	6ul
6	654 687	120bp	#6	reverse	6ul
7	690 691	#8	PCR, btm	universal	6.8ul
8	690 691	#8		reverse	6.8ul
9	691 693	#1	PCR	universal	5ul
10	691 693	#1		reverse	5ul

Denaturation Templates

Sequence with new kit (6/27/90)

denatured gel formula with Jeffs BioRad. Resin

Results

no good sequence info.

all overlapping peaks (clones 1, 2, and 9)

or "ASE 1002" error

peak width cannot be computed (remainder)

Assembly not problems with kit as John has used it successfully

7.11

000074

35S sequencing

to obtain information on plasmids
problems with AIF

To sequence:

					DNA	H ₂ O
1	654687	top #1	410 ng/lul	Universal	12.4	6
2	"			Reverse	12.4	6
3	654687	btm #6	820 ng/lul	Universal	6.1	12
4	"			Reverse	6.1	12
5	690691	#8	730 ng/lul	Universal	6.8	5.2
6	"			Reverse	6.8	5.2
7	691693	#1	1.04 ng/lul	Universal	5.1	13
8	"			Reverse	5	13
9	PCR 2 #2		465 ng/lul	Universal	10.7	7.3
10	"			Reverse	10.7	7.3
11	PCR 2 #7		740 ng/lul	Universal	6.7	11.24
12	"			Reverse	6.7	11.24

use Pharmacia Sequencing kit (Cydnic)

includes universal primer

use reverse primer F12 from AIF kit

8.1 sequencing gel

35S long

1
2
3
4
5
6
7
8
9

1/2

10

11

12

=

1 repeated

35S short

1
2
3
4
5

1/2

6

7

8

9

10

11

12

cracked gel
from plates

Results

Sequences OK

except PCR 2 #2 or #7.

Miniprep Bacteria in From Genevive - NYBC

clones	10-12 primers	#17	tissue 298	392 bp core
	10-12 primers	#43	5' cDNA	392 bp core
	15-17 primers	#26	tissue 19-16	617 bp env
	15-18 primers	#31	tissue 14-13	151 bp env
	15-18 primers	#51	cDNA 17-16	151 bp env

inoculate cultures: max prep.

PHOTOMETRY/DNASCAN4U

07/11/90 11:18

ID	ABS		
260.0nm	0.425A	3	2.125ug/L
260.0nm	0.027A	51	135ng/L
260.0nm	0.057A	17	285ng/L
260.0nm	0.157A	43	785ng/L
260.0nm	0.391A	69	1.955ug/L
260.0nm	0.076A	20	380ng/L

Primer Locations:

(a) ggcgcactccaccatagatcactccctgtgaggaactactgctcttcacgcagaaagcgctctagccatggcgcttagatgagtgctgctgcagcctccag 100
(b) -----
gaccccccccccgggagagccatagtggtctctcggaacccggtgagtacacgggaattgcccaggacgaccgggtcccttcttggataaacccgcgtcaatg 200

cctggagatttgggcgcgcccccycaagactgctagccgagtagtgttgggtcgcgaaaggccttgtggtactgcctgatagggtgcttgcgagtgcccc 300

gggaggtctctgtagaccgtgcaccctgagcaccgattcccaaacctcaaaagaaaaaccaaactgaacacaaacccgctgcgccacaggaagctcaagtgtcccg 400

gtggcggctcagatcggtgggtgagatttactgtgttccggcgcaggggcccctagattgggtgtgctgcgcgacgaggaagacttccagagcggtcgcaacctcg 500

aggtagacgtcagccctatccccaaagtgctgtcgcccgagggscaggaccctgggtctagcccgggatacccttggcccctcttatggcaatgagggctgcggg 600

tgggcgggatggctcctgtctccccgtgctctcgccctagttggggcccccacggaccccgccgctaggtcgccgaatttgggttagggtcatcgatacc 700

tcacgtgcgcttgcgcgacgtcatcgggatcacatccgctcgctcgccgcccctcttggaggcgctgcccagggccctggcgcatgctgggttctgga 800

agacggcgtgaactatgcaacagggaaaccttccgtgtgctcttctctatcttcttcttggcccctgctcttcttgcctgactgtgccccttcagcctac 900

caagtcgcgaactccacagggccttcatgtcaccgaatgattgcccctaacctcagattgtgtacagggcgacgatgcactctgcatactccgggg 1000

gtgtcgttcgcttcgcaagggcaacgtctcgagggttggtggtggcgcaccccccacggtagccagggagggcaaacctccccgcacgcagcttcg 1100

adgtcacaatcgatctgtctgttcggagagccaccctctgttgcggccctctacgtgggggattctgtgcgggtccgctcttcttattggtcaactgtttacc 1200

ttctctccagggccacatgacaaagcgaaggtgcgaattgttctatctaccccgccatataacgggcatcgcatggcatgggaatgataatgaact 1300

gggtccctacggcgggcttggtaatggctcagctgctccggatccccacaagccatcttggatatgatcgttggtgctcactggosagctctgscgggcat 1400

agcgtatttctcctagtggggaaactggggcaaggtcttgtagtgctgttgcgttggccggcgtcgacgcgggaaccatcgctctccgggggacaagcc 1500

gcccgccgcatgtcggacttctttagtctcttccacacagggcctaaagcagaacatccagctgatcaacaccaacggcaggttgcacatcaatagcacgg 1600

ccttgaaactgcaatgaaagccctaacacggctggtagcagggcttactatcaacacaaatttcaactcttccggctgtgccgagcgttggcagctg 1700

ccgacgcttaccgatttaccagggctggggccctatcagctatgccaaaggaagggcccccacacacacgcccttattgttgccacttcccccaaaa 1800

ccttggcggtatcggtgcccaagagcggtatgtggccgggtatattgcttcactcccagcccc 1963

12 JULY 90 - ALF sequencing

#	clone	primer	Denature	
			DNA	H ₂ O
1	654 687 *6	universal	6	4
2	654 687 *6	reverse	6	4
3	690 694 *9	universal	2.5	7.5
4	690 694 *9	reverse	2.5	7.5
5	690 694 *11A	universal	6.4	3.6
6	690 694 *11A	reverse	6.4	3.6
7	690 691 *21B	universal	2.5	7.5
8	690 691 *21B	reverse	2.5	7.5

Set up on ALF

old model plate - new copier (used w before)
 used new "non prototype" kit

results:

"peaks cannot be computed"

shown on 1-2 & 5-8 clones

peaks 3-4 were computed

but data is all overlapping

Potential problem shooting:

try new glass plate
 new copier

kit was used previously by John with positive re

7/12

³⁵S sequencing of plasmids

ds procedure → CYNDIES kit
³⁵S of 7/10

	clone	primer	DNA	H ₂ O
1	10.12 #17	590	18	
2	10.12 #17	591	18	
3	10.12 #17	693	18	
4	10.12 #17	694	18	
5	10.12 #43	590	6.3	11.7
6	10.12 #43	591	6.3	11.7
7	10.12 #43	693	6.3	11.7
8	10.12 #43	694	6.3	11.7
9	15.17 #26	590	13.15	4.85
10	15.17 #26	591	13.15	4.85
11	15.18 #31	590	2.35	15.65
12	15.18 #31	591	2.35	15.65

long gel:

1
2
3
4
5
6
7
8

12

9

10

11

12

-

2

3

4

old samples

short gel:

3

4

5

6

-

1

2

3

4

5

6

7

8

9

10

11

12

ad samples

4 → ran a little

12

Results. primers 653 + 654 did not work - did not arrive ?

000076

7/16 New Round AIF

Samples:

1	628.629	- universal	} Control plasmid for sequencing - new primers
2	628.629	- reverse	
3	628.629	- 702	
4	628.629	- 703	
5	690.694	#9 universal	} 3:2ul = 6.8ul H ₂ O 2:5ul = 7.5ul H ₂ O
6	690.694	#9 reverse	
7	15.17	#26 universal	} 10ul DNA
8	15.17	#26 reverse	
9	15.17	#26 702	
10	15.17	#26 703	

new FITC dyes 702 (590 BS primer) 20.5 ug/ml 3.6 μ m
 703 (591 BS primer) 51.0 ug/ml 8.96 μ m

set up of AIF

used 70 dilution (2 μ) - 0.05 inc

new front gel plate

new loader

changed to second set of nonprototype kit ingredients
 sequence reactions done in water bath at 37°

Results

16 Jul 90 AIF

1	628.629	✓	} processed data, but slightly overlapping overlapping data could not compute peak system error
2		r	
3		702	
4		703	
Export	5	690.694 - 90	} worked! 300bp sequence G's did not resolve well.
	6	91	
	7	15.17 - ✓	} some gaps - problems with A peaks trend = 0.00
	8	15.17 - r	
	9	15.17 - 702	} processed, but trend = 0.00 system error
	10	15.17 - 703	

only one clone worked, but results seem more encouraging
 than previous failed runs.

7/12 ^{35S} sequencing

To complete sequences of remaining plasmids constructed.

^{35S} sequence - use Cynclis AutoRead kit.

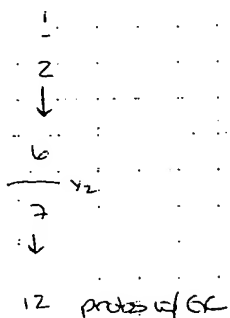
^{35S} from 7/10.

set up template denaturation ⁹⁰N - sequence, run gels next AM.

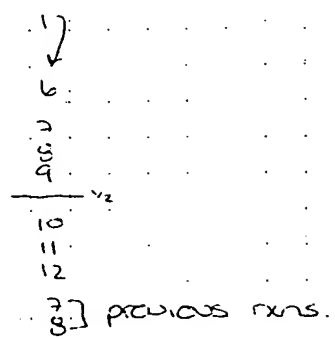
#	clone	primer	DNA	H ₂ O
1	690.694 #9	universal	2.5	15.5
2	690.694 #9	reverse	2.5	15.5
3	690.694 #11A	universal	6.5	11.5
4	690.694 #11A	reverse	6.5	11.5
5	690.691 #21B	universal	2.5	15.5
6	690.691 #21B	reverse	2.5	15.5
7	10.12 #17	690	18	
8	10.12 #17	691	18	
9	10.12 #43	690	6.4	11.6
10	10.12 #43	691	6.4	11.6
11	15.18 #51	702	18	
12	15.18 #51	703	18	

gels

long



short



NOTES on 10.12 capsid gene cloning from NYBC.

re FAX of July 11. from NYBC

Sequence shows limited homology with Japanese primers - most homology later determined to not be a correct clone.

000077

7/19/90

19 JUL 90 ALF

ALF sequencing run

done with Mark Freeman
Pharmacia NJ.

- clones:
- | | | | |
|----|-----------|---------------|---------------------|
| 1 | PUC18 | universal | } kit controls |
| 2 | PUC18 | reverse | |
| 3 | M13 | universal | |
| 4 | 690691 | ISE universal | } undies BS primers |
| 5 | 690691 | ISE reverse | |
| 6 | 15.17 *26 | 702 | |
| 7 | 15.17 *26 | 703 | |
| 8 | PUC18 | universal | |
| 9 | PUC18 | reverse | |
| 10 | M13 | universal | |

1200 volts 400s
40mA 10 clones
36W
45° → 40°
3mW

Denaturation of Templates

- A. PUC18 control 2* universal 500ng/ul =
2* reverse 10ul for Sug
- B. 690691 ISE

402ng/ul - use 10μl

did 10ul DNA C. 15.17 = 380ng/ul - use 100ul

+ 3ul 2M NaOH

+ 10ul + NaAc + EtOH according to previous pen

EtOH ppt

Did not wash w/ 70% → sequence as usual

ALF run did not work for ss or ds templates.

problems w/ ds runs; NaOH denaturation?

unlikely is same procedure for 3'SS

7/20/90

20 JUL 90 ALF

repeat of ALF sequencing
for ss M13 runs only.
check for machine working

- | | | |
|---|-----------------|---|
| 1 | } SL2 w/ 29 kit | M13 universal primer |
| 2 | | M13 [extension buffer] w/ anneal buffer |
| 3 | | M13 + T ₇ from 3'SS kit |
| 4 | | M13 |
| 5 | | M13 |

- | | | |
|----|---------------------------------|---|
| 6 | } MF. 6/29 kit. (non prototype) | M13 universal primer |
| 7 | | M13 w/o extension buffer. 1cm |
| 8 | | M13 w/o " + H ₂ O |
| 9 | | M13 X + T ₇ from 3'SS kit |
| 10 | | M13 |

Results = Run worked! NO machine / software problems

Exhibit 4, page 29

690694-92u.Seq x 690694-9u.Seq July 19, 1990 07:00 ..

269.

14 TCTAGAGGATCCCCCTCTGCCCCGCGCCGACGTGCCCTTGGGGATAGGC 63
1 TCTAGAGGATCCCCCTCTGCCCCGCGCCGACGTGCCCTTGGGGATAGGC 50
64 TGACGTCTACCTCGAGGTTGCGACCGCTCGGAAGTCTTCTTCGTGCGCG 113
51 TGACGTCTACCTCGAGGTTGCGACCGCTCGGAAGTCTTCTTCGTGCGCG 109
114 CACACCCAATCTAGGGCCCCCTGCGCGGCAACAAGTAACTCCACCAACGA 163
101 CACACCCAATCTAGGGCCCCCTGCGCGGCAACAAGTAACTCCACCAACGA 150
164 TCTGACCGCCACCCGGGAACCTGACGTCTTGTGGGCGACGGTTGGTGTTA 213
151 TCTGACCGCCA.CCGGGAACCTGACGTCTTGTGGGCGACGGTTGGTGTTA 199
214 CGTTTGGTTTTTCTTTGAGGTTTGGGAATGTGC 246
200 CGTT..GGTTTTCTTTGAGG.TTGGGAATGTGC 229

probs
w/9u

690694-92u.Seq x 690694-92r.Seq July 19, 1990 08:02 ..

92u/
92r=α

92s α
better

46 CGTGCCTTGGGGATAGGCTGACGTCTACCTCGAGGTTGCGACCGCTCGGA 95
246 CGTGCCTTGGGGATAGGCTGACGTCTACCTCGAGGTTGCGACCGCTCGGA 197
96 AGTCTTCTCTGTCGCGCGCACACCCAATCTAGGGCCCCCTGCGCGGCAACA 145
196 AGTCTTCTCTGTCGCGCGCACACCCAATCTAGGGCCCCCTGCGCGGCAACA 147
146 AGTAAACTCCACCAACGATCTGAC.GCCACCCGGGAACCTGACGTCTTGT 195
146 AGTAAACTCCACCAACGATCTGACCGCCACCCGGGAACCTGACGTCTTGT 97
193 GGGCGACGGTTGGTGTACGTTTGGTTTTCTTTGAGGTTTGGGAAT 242
95 GGGCGACGGTTGGTGTACGTTTGGTTTTCTTTGAGGTTTGGGAAT 50

690694-11au.Seq x 690694-11ar.Seq July 19, 1990 07:02 ..

31 ATTCCCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 80
238 ATTCCCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 189
81 GGACGTCAAGTCCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGT 130
188 GGACGTCAAGTCCCCGGGTGGCGGTCAG.TCGTTGGTGGAGTTTACTTGT 140
131 TGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 180
139 TGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 90
181 GAGCGGTTCGACCTCGAGGTAGACGTACGCTATCCCAAGGCACGTCTG 230
89 GAGCGGTTCGACCTCGAGGTAGACGTACGCTATCCCAAGGCACGTCTG 40
231 GCCCGAGG 238
39 GCCCGAGG 32

see next page

Sequence Organization ALF - 355 runs

000078

690694

REFORMAT of: 690694-9u.Seq check: 7228 From: 1 To: 287 July 17, 1990 10:42
(No documentation)

690694-9u.Seq Length: 287 July 17, 1990 10:42 Check: 7228 ..

1 TCTAGAGGAT CCCCCTCTCT CCCCCTGGGCC GACGTGCTT GGGGATAGGG
51 TGACGTCTAC CTCCAGGTTG CGACCGCTCG GAAGTCTTCC TCGTCGCGCG
101 CACACCCAAT CTAGGGCCCC TCGCGGSCAA CAAGTAAACT CCACCAACGA
151 TCTGACCGCC ACCGGGAAGT TGACGTCTCT TGGCGACGG TTGGTGTAC
201 GTTGGTTTTC TTGAGGTTG GGAATGCT CATGGGTACG AGCTCGAATC
251 gtaacagtc aagcgtttcc tctgtgaaatg ttaaa

690694-92u.Seq Length: 246 July 18, 1990 14:30 Check: 298 ..

1 CCTGCAGGTC GACTCTAGAG GATCCCCGTC CTGCCCTCGG GCCGACGTGC
51 CTTGGGGATA GGCTGACGTC TACCTCGAGG TTGCGACCGC TCGGAAGTCT
101 TCCTCGTCGC GCGCACACCC AATCTAGGGC CCTGCGCGG CAACAAGTAA
151 ACTCCACCAA CGATCTGACC GCCACCCGGG AACTTGACGT CCTGTTGGCG
201 ACGGTITGGT TTACGTTTGG TTTTCTTTG AGGTTTGGGA ATGTGC

690694-92r.Seq Length: 220 July 18, 1990 14:34 Check: 5194 ..

1 CGAGCTCGGT ACCCATGAGC ACGATTCCCA AACCTCAAAG AAAAACCAAA
51 CGTAACACCA ACCGTCGCCC ACAGGACGTC AAGTCCCCGG GTGGCGGTCA
101 GATCGTTGGT GGAGTTTACT TGTTCGCGG CAGGGGCCCT AGATTGGGTG
151 TCGCGCGGAC GAGGAAGACT TCCGAGCGGT CGCAACCTCG AGGTAGACGT
201 CAGCCTATCC CCAAGGCACG

690694-113u.Seq Length: 238 July 18, 1990 14:40 Check: 9399 ..

1 GGTCGACGCT AGAGGATCCC CATGAGCAGC ATTCCCAAAC CTCAAAGAAA
51 AACCAAACGT AACACCAACC GTCGCGGACA GGACGICAGG TTCCCGGGTG
101 GCGGTCAGAT CGTTGGTGGG GTTACTTGT TGCCGCGCAG GGGCCCTAGA
151 TTGGGTGTGC GCGCGACGAG GAAGACTTCC GAGCGGTTCG AACCTCGAGG
201 TAGACGTGAG CCTATCCCCA AGGCACGTCG GCCCGAGG

690694-114r.Seq Length: 225 July 18, 1990 14:44 Check: 3275 ..

1 GCTCGGATCC GTCCTGCCC TGGGCGGAC GTGCTTGGG CATAGGCTGA
51 GGTCTAGCTC GAGGTTGGA CCGCTCGGAG GTTCTCTCG TCGCGCGAC
101 ACCCAATCTA GGGCCCTGCG GCGGCAACGA GTAACTCCA CCAAGGACTG
151 ACCGCCACCC GGGAACTTGA GGTCTCTGCG GCGACGTTG GTGTTACGTI
201 TGGTTTCTT TTGAGGTTTG GGAAT

690694 - continued

690694-11au.Seq x 690694-92u.Seq July 19, 1990 07:05 ..

seq of
92u=oc
match to
11A

```
31 ATTCCTCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 80
   |||||||
234 ATTCCTCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 185
   |||||||
81 GGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGT 130
   |||||||
184 GGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGT 135
   |||||||
131 TGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 180
   |||||||
134 TGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 85
   |||||||
181 GAGCGGTGCGCAACCTCGAGGTAGACGTACGCTATCCCCAAGGCACGTGG 230
   |||||||
84 GAGCGGTGCGCAACCTCGAGGTAGACGTACGCTATCCCCAAGGCACGTGG 35
   |||||||
231 GCGCGAGG 238
   |||||||
34 GCGCGAGG 27
```

Hcv-Core.Seq x 690694-92u.Seq July 19, 1990 07:03 ..

= only
changes
with HCV

→ 690

```
10 attcccaaacctcaaaagaaaaaccaaaccgtaacaccaaccgtagccacac 59
   |||||||
376 ATTCCTCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 327
   |||||||
60 ggacgtcaagttcccggtggcggtcagatcggtgggtgggtttacttgt 109
   |||||||
326 GGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGT 277
   |||||||
110 tgccgcgccagggggccctagattgggtgtgcgcgcgacgaggaagacttcc 159
   |||||||
276 TGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 227
   |||||||
160 gagcggtcgcaaacctcgaggttagacgtacgctatccccagggtagctcg 209
   |||||||
226 GAGCGGTGCGCAACCTCGAGGTAGACGTACGCTATCCCCAAGGCACGTGG 177
   |||||||
210 gcccgggggagggacctgg 228
   |||||||
176 GCGCGAGGCGAGGACGGGG 158
```

Hcv-Core.Seq x 690694-9u.Seq July 17, 1990 08:06 ..

690

```
1 atgagcagcattcccaaacctcaaaagaaaaaccaaaccgtaacaccaaccg 50
   |||||||
226 ATGAGCAGC.ATTCCC.AACCTCAAACAAAAA..CCAACTAACACCAACCG 281
   |||||||
51 tgcccccagggagcgaagttcccggtggcggtcagatcggtgggtgggt 100
   |||||||
280 TGCCCGCAGGAGCGTCAAGTTCCC.GGTGGCGGTCAGATCGTTGGTGGAG 332
   |||||||
101 ttacttgtagccgagcagggggccctagattgggtgagcgcgagcagag 150
   |||||||
231 TTTACTTGTGTGCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGG 182
   |||||||
151 aagacttcgagcggtcgcaaacctcgaggttagacgtacgctatccccaa 200
   |||||||
181 AAGACTTCCGAGCGGTGCGCAACCTCGAGGTAGACGTACGCTATCCCCAA 132
   |||||||
201 ggtgagtcgaggggagggacctgg 226
   |||||||
131 GGCACGTGCGCCGAGGGGAGGACGGGG 104
```

probably
this 9u seq
see previous
page

691693 clone

only one isolated & sequenced = #1

00007

691693-1u.Seq Length: 280 July 17, 1990 13:13 Check: 8434

1 TTGCATGCTT GCAGGTCGAC TCTAGAGGAT CCCCATTCAC GTCCGCCCGA
51 GGGCAGGACC TGGGCTCAGC CCGGTACCTT TGGCCCTCT ATGGCAATGA
101 GGGTTCGGG TGGGCGGGAT GGTCTCTGTC TCCCCGTGGC TCTCGGCTA
151 GCTGGGGCCC CACAGACCCC CGGCGTAGGT CGGCAATTT [✓]GGGTACCGAG
201 CTGGAATTTC TAATCATGTC ATAGCTGTTT CCGTGTGAA ATTGTTATCC
251 GCACACAATT CCACACAACA TACGAGCCGG

691693-1r.Seq Length: 195 July 17, 1990 13:16 Check: 400

1 TTCGAGCTCG [✓]GTACCCAAAC GCGACCTACG CCGGGGGTCT GTGGGGCCCC
51 AGCTAGGCCG AGAGCCACGG GGAGACAGGA GCCATCCCGC CCACCCGCAA
101 CCTCATTTGC CATAGAGGGG CCAAGGGTAC CCGGCGTGA GCCAGGTCT
151 GCCCACGGGC CGACGTGCTT [✓]GGGATCCTC TAGAGTCGAC CTGCA

691693-1u.Seq x 691693-1r.Seq July 17, 1990 13:05

10 TGCAGGTGACTCTAGAGGATCCCA [✓]TCAGGTGCGGCCCG [✓]GGCAGGAC 59
230 TGCAGGTGACTCTAGAGGATCCCA [✓]AGCAGCTCGGCCCG [✓]GGCAGGAC 231
60 CTGGGCTCA [✓]CCGGGTACCTTGGGCCCTCTATGGCAATGAGGGTTC 107
230 CTGGGCTCA [✓]CCGGGTACCTTGGGCCCTCTATGGCAATGAGGGTTC 183
108 GGGTGGGCGGGATGGCTCTCTCTCCCGTGGCTCTCGGCTAGCTGGGG 157
181 GGGTGGGCGGGATGGCTCTCTCTCCCGTGGCTCTCGGCTAGCTGGGG 130
158 CCCCACAGACCCCCGGGTAGGTGCGCAATT [✓]GGGTACCGAGCTCGAA 206
131 CCCCACAGACCCCCGGGTAGGTGCGG... [✓]TTGGGTACCGAGCTCGAA 84

check on seq films.

with corrections to
691693-1u.

Hcv-Core.Seq x 691693-1u.Seq July 17, 1990 18:07

172 cctcggggtgagcgtcagcctatccccgagggtgagtcggcccgaggagg 221
8 CCTGCAGGTGACTCTAGAGGATCCCA [✓]ATTC [✓]ACGTCGGGCCCGAGGGCAG 51
222 gacctggggtcagcccggtacccttggcccccctatggcaatgagggct 271
57 GACCTGGGCTCAG [✓]CCGGGTACCTTGGGCCCTCTATGGCAATGAGGGIT 107
272 gagggtggggtgaggtgactcctgtctccccgaggctctcagccctanttg 321
106 GCGGGTGGGCGGGATGGCTCTCTCTCCCGTGGCTCTCGGCTAGCTGGGG 157
322 gggcccgaggacccccgggtagggtcgggctatgggaggggtatgaa 157
156 GGGCCACAGACCCCCGGGTAGGTGCGCAATT [✓]GGGTACCGAGCTCGAA 206

= confirmed
changes w/ HCV

clone is not full
length 693-691
stops past the
694 ntiles

NOTE - 49-CA Δ is
confirmed

1' ¹⁵GGTCATCGATACCCTTACGTGCGGCTTCG⁵⁶ACCTCATGGGGTACATAC 50
4 GGTCA¹⁶TCGATACCCTTACGTGCGGCTTCGACACCTCATGGGGTACATAC 53
51 CGCTCGTCGGCGCCCTCTTGGAGGCGTGCCAGGGCCCTGGCGCATGGC 100
54 CGCTCGTCGGCGCCCTCTTGGAGGCGTGCCAGGGCCCTGGTGCATGGY 103
101 GTCCGGGTTCTGGAAGACGGCGTGA¹⁷ACTATGCAACAGGGAACTTCCCAG 150
104 GTCCGGGTTCTGGAAGGCGGCGTGGGCTATGCGACAGGGGGCCTTCCCAG 153
151 ¹⁸TTGCTCTTTCTCTATC 166
154 TTGCTCTTTCTCTATC 169

```

37  TTCGCGCACCTCATGGGGTACATACCGCTCGTCGGCGCCCTCTTGGAGG 86
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
220  TTCGCGCACCTCATGGGGTACATACCGCTCGTCGG. GCCCCTCTTGGAG. 173
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
87  CCGTGCCAGGGCCCTGGCGCATGGCGTCCGGGTCTCTGGAAGACGGCGTGA 136
   CAGT  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
172  .CGCTCCAGGGCCCTGG. GCATGGCGTCCGGGTCTCTGGAAGACGGCGTGA 125
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
137  ACTATGCAACAGGGAA. CCTTCCCA. GTTGCTCTTTCTCTATCTGGGGGA 184
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
124  ACTATGCAACAGGGAATCCTTCCCA GTTGCTCTTTCTCTATCTGGGGGA 75
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
185  TCCACTAGTTCTAGAGCGGCCGCCACCGC. GTGGAGC 220
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
74  TCCACTAGTTCTAGAGCGCGCCACCGCGGGTGGAGC 38
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||

```

use 702: NYBC sequence 1st = F version.
then compare: A with 1518, 51703

clone; SI results \rightarrow NYBC
only homology = w/ primer
SEC FAX of July 11.

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